FOOD AS A FACTOR IN THE DETERMINATION OF SEX IN AMPHIBIANS.

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Of the many theories that have been advanced regarding the causes that determine whether an animal shall become male or female, the one that nutrition is a dominant factor in sex determination has received much credence. This theory has been supported by the results of numerous feeding experiments made by different investigators on various classes of animals, and also by statistics compiled by Düsing (5) and others with reference to the proportion of males and females in the human race among the offspring of the rich and of the poor.

Three investigators, Born, Yung and Cuénot, have sought by experimental means to ascertain the relation of nutrition to sex determination in amphibians. Born (1), who was the first investigator in this field, found that in a total of 1,272 young *Rana fusca* that had been well nourished during the larval period, 1209 or about 95 per cent. were females, while in 160 young frogs taken from their natural environment only 52 per cent. were females. From the results of these experiments Born concluded that an abundance of food leads to the development of a greater proportion of females. As several investigators have pointed out, Born's results cannot be considered as furnishing conclusive evidence regarding the influence of nutrition on sex determination, for the methods employed in the experiments did not exclude the possibility that other factors than nutrition influenced the results. No account whatever was taken of the many hundreds of tadpoles that died during the course of the investigations and, as Born himself suggests, there is the possibility that the mortality was greater among the males than among the females. In ascertaining the sex of the young frogs, Born examined the gonads *in toto* and did not make use of sections in any case: if the genital organs were large, the individual was classed as a female; if the organs were small, the individual

was considered to be a male. Such a method of distinguishing the sexes in young frogs has been found to be unreliable, as at the time of metamorphosis the genital organs are not very well developed and it is often impossible to determine the sex of an individual with any degree of certainty without making a histological examination of the gonads.

The experiments of Yung (13) on *Rana esculenta* were made, primarily, to study the influence of various kinds of food on the development of the tadpoles, but the results seem to furnish positive evidence that the sex of *Rana* is influenced by nutrition. Yung's experiments were carried out with great care, the different lots of eggs being kept under similar external conditions and the food alone differing in the various cases. In considering his results, Yung also failed to take into account the tadpoles that died during the course of the experiments, and he ascertained the sex of only those individuals that underwent metamorphosis. In these experiments the number of females that developed varied from 70 per cent. to 75 per cent. in different cases, the greatest number being found among the lot of frogs that had received only animal food. In a later series of experiments Yung (14) found that in a lot of 100 young frogs that had been fed exclusively on beef, 78 per cent. were females; the number of females was found to be increased to 81 per cent. in a second lot of 100 tadpoles that had been fed on fish; while in a third lot of 100 tadpoles that had received the flesh of frogs as food the number of females was 92 per cent. From an investigation of the sex of 300 young *Rana esculenta* that had developed under natural conditions, Yung concluded that normally the number of females in this species of *Rana* is about 53 per cent. The results of Yung's experiments, therefore, support Born's conclusion that nutrition is a decisive factor in sex determination, an abundance of food leading to the development of a large proportion of females.

In a recent paper, Cuénot (3) gives the results of a series of feeding experiments which he made on the larvæ of *Rana temporaria* in order to test the conclusion reached by Born and Yung. Cuénot's results do not agree with those obtained by the earlier investigators, as in two lots of frogs that had been well nourished on animal food he found an excess of males; while in another

lot of frogs that had been poorly nourished, there was a greater proportion of females. Cuénot states that, as the results of all of the feeding experiments that have been made on *Rana* are contradictory, it is evident that nutrition is not an absolutely dominating factor in sex determination. He believes that there is a strong probability that sex is already determined in the egg at the time of deposition.

As Cuénot used comparatively few individuals in his experiments and as his results do not accord with those obtained by Born and Yung, it is obvious that the question of the influence of nutrition in determining the sex of amphibians is still an open one. It is necessary, therefore, that many more experiments should be carried out along the lines suggested by the work of these investigators.

At the anterior end of the genital organs in the tadpoles of the common American toad, *Bufo lentiginosus*, there is found a small rounded structure, the so-called "Bidder's organ" (Fig. 1, *B*), which is composed apparently of undeveloped ova. The function of this organ is unknown, and whether it is a rudimentary ovary, as many investigators have maintained, has not as yet been satisfactorily determined. This body is found in young tadpoles some time before it is possible to distinguish sex; and it is a permanent organ in the male, disappearing in the female near the end of the second year. If Bidder's organ proves to be a rudimentary ovary, then the adult male toads are in a sense hermaphrodites, although the same cannot be said of the adult female unless the male elements are present in some form that as yet has not been discovered. Because, therefore, of a possible condition of hermaphroditism in the young tadpoles, which might seem to indicate that sex is not already determined at this stage of development, *Bufo lentiginosus* was chosen as more favorable material than any common species of *Rana* for an investigation of the influence of external factors on sex determination. As the tadpoles of *Bufo* are somewhat smaller than those of *Rana* and are easily reared under artificial conditions, they are well adapted for experiments that must, of necessity, extend over a considerable period of time.

The present paper records the results of the first of a series of

experiments which have been planned in the hope that it may be possible to show whether external factors such as temperature, nutrition, time of fertilization, etc., have any influence in determining sex in *Bufo*. Recent investigations on other forms have

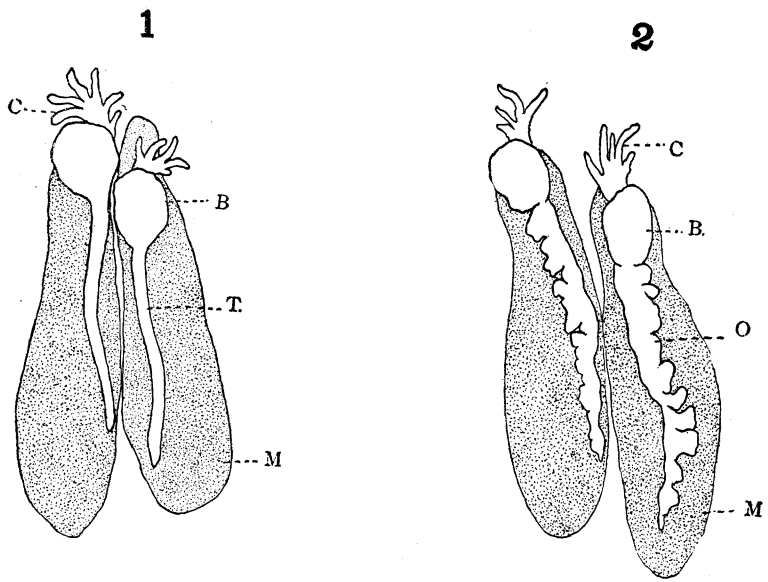

FIG. 1. Camera drawing of the genital organs of a male toad killed soon after matamorphosis. *T*, testis; *B*, Bidder's organ; *C*, corpus adiposum; *M*, kidneys.

FIG. 2. Camera drawing of the genital organs of a female toad killed soon after metamorphosis. *O*, ovary. Other lettering as in Fig. 1.

seemed to indicate that sex is not influenced by external conditions, but that it is determined either before or during the fertilization of the egg. If this is indeed the case, experiments such as those that I have in mind will yield only negative results. But if it can be shown for even one form that external factors may be disregarded in considering the question of sex determination, something will have been gained towards an ultimate solution of the problem.

METHOD.

In endeavoring to ascertain the part played by any one factor in sex determination, it is, of course, absolutely necessary that the influence of all other factors shall be eliminated as far as possible. In making the experiments recorded in the present paper,

very great care was taken that all of the individuals being experimented upon were kept under similar external conditions, the only factor that was intentionally varied being that of nutrition whose action it was proposed to study.

Two separate series of experiments were made which, for convenience, will be called Series I., and Series II. The eggs used in Series I., were laid in the laboratory and normally fertilized on the morning of April 13, 1906; while those used in Series II. were laid under similar conditions on April 16, 1906. Both lots of eggs were kept in large aquaria until the tadpoles hatched, and the experiments began in each case five days after the eggs were laid.

Series I. was started with a total of 1,500 individuals; but, as will be explained later, only 1,100 of these can be taken into account in considering the results. Eight hundred individuals were used in the second series of experiments, making a total of 1,900 individuals upon which to base conclusions from the results obtained. Glass dishes of uniform size were used throughout the experiments, each dish containing, in the beginning, 100 tadpoles. The dishes were kept together so that the tadpoles were all under the same conditions of temperature. The water used was "tap" water obtained from an artesian well and used for drinking purposes, so presumably it was free from unicellular organisms. Approximately the same quantity of water was kept in each dish.

Out of the total of 1,900 individuals, only 364, or 19.15 per cent. died before it was possible to ascertain the sex. This comparatively low rate of mortality during the early stages of development I attribute in great part to the fact that the water in the dishes was never allowed to become foul. When the tadpoles were very small the water was changed on alternate days and the dishes carefully cleaned. Later, as the tadpoles became larger, it was necessary to change the water every day. During some very warm weather in June when the tadpoles were beginning to undergo metamorphosis, the water was renewed as often as four or five times daily.

In *Bufo* the genital organs are apparently much better developed at the time of metamorphosis than they are in *Rana*, as in the majority of individuals it is possible to distinguish the males

from the females with absolute certainty without making a histological examination of the gonads. The method used to distinguish the sexes in very young toads was as follows: the toad was placed in a flat, shallow dish containing a layer of paraffine which makes an excellent surface for cutting, and the body cavity was then opened under a dissecting lens; the kidneys and the genital organs attached to them were removed by means of small, sharp knives, and subsequently, under a much stronger lens, the gonads were examined *in toto*. Figs. 1 and 2 show the differences between the gonads of the two sexes in young toads that have recently completed metamorphosis. The testes (Fig. 1) are at this time about 2 mm. in length, they are relatively narrow, cylindrical bodies with a smooth outline; the ovaries (Fig. 2), on the contrary, are usually broader than the testes and they have an irregular, jagged outline. Bidder's organ (Figs. 1 and 2, *B*) is very prominent in all individuals at this time; but as it is practically the same size in both sexes, it is of no aid in distinguishing males from females.

In order to ascertain whether the external appearance of the genital organs (as shown in Figs. 1 and 2) is a positive indication of the sex of the individual, 50 young toads were selected of which 25 had gonads approximately like those shown in Fig. 1, and 25 had gonads similar to those in Fig. 2. The gonads were stained *in toto* with hæmatoxylin and sectioned. The histological examination proved conclusively that the external appearance of the gonads can be relied on to indicate the difference in sex, as the sections showed unquestionably that there were 25 males in the one lot and 25 females in the other. At the time of metamorphosis the genital organs are not equally well developed in all individual, however, and occasionally it is impossible to distinguish the sex of a toad without making use of sections.

All of the tadpoles that died during the course of the experiments were fixed in corrosive-acetic (5 per cent. acetic acid) if the hind legs were well developed and the sex ascertained, when possible, by means of sections. A histological examination of the gonads enables one to ascertain the sex of a tadpole some time before the front legs have appeared; for, although the germ-cells may appear similar at this time, the ovary has a central cavity

which is not present in the testis. Altogether the gonads of about 600 individuals were examined histologically and in only about 50 cases was it impossible to distinguish one sex from the other.

The methods used in carrying out the experiments and in ascertaining the sex of the individuals have been given in considerable detail in order to indicate the precautions that were taken to avoid the most probable sources of error that might have had an influence on the results.

The Normal Proportion of the Sexes in Bufo lentiginosus.

Cuénot has collected the statistics that have been published regarding the normal proportion of the sexes in various species of *Rana*, and his table shows that the number of females varies from 49 per cent. to 86.8 per cent. in different cases. Pflüger (9) and von Griesheim (7), who have most carefully investigated this subject, find that not only does the proportion of females vary somewhat in lots of frogs taken from different localities, but that there is also a marked difference in the proportion of females in lots of frogs taken from the same locality in different years. The normal proportion of the sexes in *Rana* seems, therefore, to be a variable one depending on the locality and on the year. In the great majority of cases there seems to be a greater number of females than of males, not only among adult frogs but also among the young just after metamorphosis: the excess varies from 1.05 per cent. to 73 per cent. in different cases.

I have not been able to find any statistics regarding the normal proportion of the sexes in other amphibians. Fischer-Sigwart (6) has noticed an excess of males among *Hyla aborea* during the breeding season, and Boulenger (2) has stated that there is an excess of males among the common European toads, *Bufo vulgaris* and *Bufo clamata*: neither investigator gives any statistics in support of his statement. For some years past I have been collecting adult toads during the breeding season and also during the summer months, and I have always found an excess of males in this species. Unfortunately I have kept no records regarding the proportion of the sexes among adults.

In order to determine the relative proportion of the sexes in young toads that have recently completed their metamorphosis,

500 individuals were collected one morning from the bank of the Susquehanna River at Owego, N. Y., during the latter part of June, 1904. The sex of each individual was ascertained by the method described above, it being necessary to make a histological examination of the gonads in only about twenty cases. The result of the investigation is summarized by hundreds in the following table.

TABLE I.

Number of Individuals.	Males.	Females.
100	51	49
100	44	56
100	46	54
100	48	52
100	52	48
500	241	259

Of the total of 500 individuals, 259 or 51.8 per cent. were females, and 241 or 48.2 per cent. were males. In *Bufo* the excess of females among the young seems to be somewhat less than that among young frogs, as according to an investigation made by von Griesheim of the sex of 440 young *Rana fusca*, 280 or 63.7 per cent. were females.

Although in the adult state the female toad is noticeably larger than the male, it is not possible to distinguish the sex of very young toads by their size alone. Two hundred individuals in this group were sorted according to size and it was found that, in many cases, the larger individuals were males. Any variation that may exist in the size of the individuals at the time of metamorphosis can probably be attributed to the difference in the amount of food that the tadpoles were able to obtain.

EXPERIMENTS.

If food is a decisive factor in sex determination, it may be considered to act in one of two ways: either through the quantity of nourishment that it affords the organism; or through its particular chemical nature as a proteid, a hydrocarbon, etc. An abundant nutrition is held by many investigators to lead to the development of an excess of females; while, on the other hand, scarcity of food, according to Schenk (10) and others, tends to

produce relatively more males. Yung maintains that nitrogenous food is highly favorable to the development of females ; while Schultze (11) states that food of this character has no influence whatever in determining sex.

It was intended, when the experiments began, to test both of the possibilities mentioned above. Among the 300 individuals of Series I., that were poorly nourished the mortality was so great during the first month of the experiment that it was necessary to abandon, for the time, the study of the possible influence of malnutrition on sex determination. The investigations were therefore confined to an attempt to ascertain whether an abundant nutrition or the character of the food received by the larvæ has any influence in determinating sex. The lot of 300 tadpoles which had received little food was therefore discarded, and all of the remaining individuals received an abundance of the particular kind of food whose influence was being investigated.

In Series I., 300 tadpoles (Lot A) were fed exclusively on a meat diet consisting of small pieces of cooked lamb or beef; 300 tadpoles (Lot B) were nourished on a purely vegetable food consisting of a cooked wheat cereal ; a third set of 300 individuals (Lot C) received a mixed diet composed of water plants (*Nitella* and *Spirogyra*) and minute organisms on decayed leaves and bits of wood taken from a pond in which toads breed each spring. Lot C presumably received food similar in character to that normally obtained by amphibian larvæ.

According to experiments made by Danilewsky (4), lecithin has a marked influence on the development of frog embryos : tadpoles fed on it show a great increase in size and in weight over control tadpoles that have not received lecithin as food. Danilewsky's experiments were not continued until the tadpoles underwent metamorphosis, and therefore his results do not indicate whether the increase in the size of the tadpoles was due to a more rapid development or whether it was the direct effect of the lecithin in producing abnormally large individuals. As it is conceivable that a more rapid development or an abnormal increase in size might possibly be factors that would influence the sex of an individual, a fourth set of 300 tadpoles (Lot D) in Series I. were fed exclusively on the yolk of hen's egg which, according

to Gautier, contains from 8.43 per cent. to 10.72 per cent. of lecithin. No attempt was made to feed tadpoles on lecithin alone, because in experiments which I made several years ago the mortality among tadpoles that were given lecithin as food was exceedingly great; the individuals dying evidently of starvation, as they were never seen to eat any of the lecithin. Owing to an accident, 100 tadpoles fed on the yolk of egg were killed the second week of the experiment. Lot D, therefore, consisted of only 200 individuals, making a total of 1,100 individuals that are to be taken into account in considering the results of the experiments in Series I.

In order to make possible a comparison between the results obtained in Series I. and those from similar experiments on the eggs of a different female, a second series of experiments were made beginning three days later than those in Series I. These experiments were similar in all respects to those in the first series, except that no attempt was made to investigate the possible influence of a scarcity of food in determing sex, and only 200 tadpoles were used in each lot. Series II. therefore consisted of 800 individuals.

Although detailed observations were made on each lot of tadpoles at intervals of about one week, only the record of Series I. for June 7, will be given. This record will serve to show the differences between the individuals of the various lots that can probably be ascribed to the varied character of the food that the tadpoles received.

Lot A. — The tadpoles fed exclusively on meat were noticeably larger than those fed on any other kind of food. The largest individuals measured 27 mm. in length, thus exceeding, by 3–4 mm., the length of a number of tadpoles of about the same age that had been reared under natural conditions; the smallest individuals in this lot were 19 mm. long and were much larger than many of the tadpoles in the other lots. Many of the meat fed tadpoles had very well developed hind legs at this time, but the front legs had not appeared in any individual as yet. It was noticed that these tadpoles were very much blacker than any of the other tadpoles being experimented upon. A meat diet is evidently as favorable to the development of pigment

in the toad tadpole as it is in the Mexican axolotl according to the observations of Shufeldt (12). The mortality in this lot was very low, only 18 individuals having died at the time the record was made.

Lot B. — The tadpoles fed on wheat were, as a whole, considerably smaller than those fed on meat, and they were more uniform in size, the greatest number having a length of about 22 mm. By June 7, three individuals in this lot had begun their metamorphosis, and 38 individuals had died.

Lot C. — The smallest and least developed tadpoles were those that were fed on a mixed diet. The greatest extremes in size were also found in this lot, the body length varying from 10–21 mm. in different cases. In many individuals the hind legs were only just visible, and in the largest individuals they were poorly developed as compared with those of the individuals in other lots. The mortality in this lot was very great, 97 individuals having died by June 7.

Lot D. — The great majority of the tadpoles fed on the yolk of egg were intermediate in size between those fed on meat and those that had received a purely vegetable diet, the average length of these tadpoles being 22–24 mm. The individuals in this lot had developed much more rapidly than those in any of the other lots. By the seventh of June, 8 individuals had begun metamorphosis and many more were on the point of doing so. The mortality in this lot also was very great as 63 individuals had died.

The differences between the individuals in the various lots of Series II. were of the same character and as strongly marked as were those in Series I. The death rate in Series II. was practically the same as in Series I.; the fewest deaths occurring among the tadpoles that were fed on meat and on cereal, the greatest number among those that were nourished on a mixed diet and on the yolk of egg.

Although the tadpoles began to undergo their metamorphosis during the first week in June, the experiments were continued until the middle of July as there was a considerable variation in the rate of development among the tadpoles of the same lot. On July 13, all of the tadpoles still living were fixed in corrosive-

acetic, as they had reached a stage of development when it would be possible to ascertain the sex of each individual by means of a histological examination of the gonads.

In the corresponding lots of the two series of experiments there was a remarkable uniformity in the rate of development of the individuals. In both series the tadpoles fed on the yolk of egg underwent their metamorphosis much sooner than any of the others, the last one in Series II. completing its metamorphosis on July 11. These tadpoles were only of average size, and none of them ever reached the length attained by many of the tadpoles that were fed on meat. Lecithin, therefore, may cause a more rapid development, but it does not produce individuals of unusual size. The tadpoles fed on meat grew enormously but this increase in size was not accompanied by a more rapid development; on the contrary, the development of these tadpoles seemed to be greatly retarded and some 50 of them had not begun metamorphosis by the middle of July. According to Yung, a purely vegetable diet is insufficient to transform a frog tadpole into a frog. Such a diet does not seem to be equally injurious to toad tadpoles, however, as comparatively few of the individuals that were fed entirely on wheat died during the course of the experiments, and only about 25 of them had not undergone metamorphosis by July 13.

As presumably the individuals that were fed on a mixed diet received the kind of food that is obtained by tadpoles living under natural conditions, it might be expected that these individuals would be larger and stronger than the others and that they would undergo metamorphosis more quickly than those receiving food that is only exceptionally, if ever, obtained by tadpoles in a state of nature. Much to my surprise the development of the individuals in Lot C lagged behind that of the tadpoles in the other lots, and large numbers of them died during the course of the experiments. On July 13, there were at least 100 tadpoles in Lot C that had not yet begun their metamorphosis.

The sex of all of the individuals used in the experiments was ascertained when possible. The results for Series I. are summarized in the following table.

TABLE II.

Character of Food Given.	Total Number of Individuals.	Sex Not Ascertained.	Males	Females.	Per Cent. of Females.	Total Sex Ascertained.
Meat (Lot A).	300	17	146	137	48.40	283
Wheat (Lot B).	300	38	119	143	54.58	262
Mixed food (Lot C).	300	108	103	89	46.35	192
Yolk of egg (Lot D).	200	49	55	96	63.57	151
Total.	1100	212	423	465	52.36	888

Table III. summarizes the results for Series II.

TABLE III.

Character of Food Given.	Total Number of Individuals.	Sex Not Ascertained.	Males.	Females.	Per Cent. of Females.	Total Sex Ascertained.
Meat (Lot A).	200	19	72	109	60.22	181
Wheat (Lot B).	200	16	76	108	58.69	184
Mixed food (Lot C).	200	43	86	71	45.22	157
Yolk of egg (Lot D).	200	74	56	70	55.55	126
Total.	800	152	290	358	55.24	648

The first conclusion that can be drawn from the above tables is that abundant nutrition alone is not a decisive factor in sex determination in *Bufo*, as in three cases (Series I., Lot A, Lot C; Series II., Lot C) more males than females were produced although all of the tadpoles had been well supplied with food during the entire course of the experiments.

As the tables show, the results of the two series of experiments in which the tadpoles were fed exclusively on meat are not in agreement. In Lot A of Series I., only 48.4 per cent. of the individuals in which sex was ascertained were females; while in the corresponding lot in Series II. there were many more females than males (20.44 per cent.). This result does not support Yung's contention that an excess of nitrogenous food leads to the development of a greater proportion of females, and it seems to indicate that food of this character has no influence in determing sex in *Bufo*. Again more rapid growth, as shown in the case of the tadpoles that were fed on the yolk of egg, cannot be considered as favoring the development of one sex any more than the other; for although in both series there was an excess of females in Lot D, this excess varies considerably in the two series (8.02 per cent.)

and is not sufficiently great in either case to warrant the conclusion that sex has been influenced by the rapid development due to the character of the food. The tables show also that a strictly vegetable diet has seemingly no influence on sex determination in *Bufo*. The slight excess of females in Lot B of each series is but little more than that which, according to my investigations, is the normal excess for the species, and it is therefore well within the limits of possible normal variation. In both series the development of the tadpoles that were nourished on a mixed diet (Lot C) was, for some unknown reason, considerably retarded and the individuals that completed metamorphosis were, as a rule, smaller than those of any of the other lots. Both series gave an excess of males in Lot C. This excess, however, is not great enough to justify the assumption that a slow development tends to produce a greater proportion of males, any more than the excess of females among the tadpoles fed on the yolk of egg warrants the conclusion that rapidity of growth favors the development of a greater proportion of females.

The results of these experiments, therefore, seem to show that the character of the food received by the tadpoles is not in itself a decisive factor in determining sex in *Bufo*, although it has much to do with the rate of development and with the size of the individuals.

The results of the experiments as given in Tables I. and II. are summarized in Table IV.

TABLE IV.

Character of Food Given.	Total Number of Individuals.	Sex Not Ascertained.	Males.	Females.	Per Cent. of Females.	Total Sex Ascertained.
Meat.	500	36	218	246	53.01	464
Wheat.	500	54	195	251	56.27	446
Mixed food.	500	151	189	160	45.84	349
Yolk of egg.	400	123	111	166	59.92	277
Total.	1900	364	713	823	53.58	1536

Of the total of 1,536 individuals in which sex was ascertained, 823 or 53.58 per cent. were females. The excess of females, therefore, is but 1.7 per cent. more than the normal excess as ascertained by the examination of the sex of 500 young toads

which had developed under natural conditions. The number of females is greatest in the lot of tadpoles fed on the yolk of egg, being 8.1 per cent. above the normal; and it is least in Lot C where it falls 5.9 per cent. below the normal. These figures are, however, well within the limits of possible normal variation for the frog as determined by the investigations of Pflüger and Griesheim, and presumably, therefore they are also within the limits of normal variation in *Bufo*.

It has been suggested by Born, and emphasized by other investigators (Cuénot, Morgan (8)) that the results obtained in feeding experiments may possibly be influenced by the mortality that occurs during the course of the experiments, individuals of one sex dying more readily than those of the other. During the course of my experiments from 30–150 individuals in each lot died before metamorphosis. These individuals, as I have stated, were preserved and the sex ascertained when possible by means of sections. From the records that were made it appears that tadpoles of one sex did not die in greater numbers than those of the other. In the entire number of individuals that were examined the proportion of the sexes was practically the same; in some lots the females died in greater numbers than the males, while in other lots the reverse was the case. These results confirm Pflüger's contention that there is no relation whatever between mortality and sex among tadpoles reared under artificial conditions.

Taking into consideration the entire number of individuals used in the experiments, it is found that in the total of 1,900 tadpoles, 823 or 43.31 per cent. developed into females; 713 or 37.52 per cent. became males; leaving 364 or 19.15 per cent. in which the sex of the individuals was not ascertained. If we assume, for the moment, that all of the individuals belonging to this 19.15 per cent. would have developed into females (although the investigation of the sex of the individuals that died during the course of the experiments does not warrant such an assumption), the number of females would then be increased to 1,187 or 62.47 per cent. of the whole number of individuals; on the other hand, if all of the in dividuals in which the sex was not ascertained had developed into males, then the number of males would be 1,077

or 56.68 per cent. of the whole number of individuals. On neither of these assumptions is the proportion of the sexes in the 1,900 individuals changed sufficiently to justify the conclusion that the nutrition has any influence in the determination of sex in *Bufo*. The results of these experiments, taken as a whole or in part, seem to show that sex is not determined either by the quantity or by the quality of the food that the larvæ receive. This conclusion agrees essentially with that reached by Cuénot from the results of his investigations on frogs, moths and other forms, and by Schultze from his experiments on mice.

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